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THE INFLUENCE OF THE INFUSION AND EXTRACTS OF *SYMPHYTUM OFFICINALE* LINN LEAVES ON THE ISOLATED GUINEA PIG UTERUS

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ABSTRACT

Infus dan beberapa ektrak daun Symphytum officinale Linn yang telah diidentifikasi dari Balai Penelitian Tanaman Obat, Tawangmangu telah diperiksa terhadap uterus marmut terisolasi.

Semua bahan bersifat uterotonik (merangsang uterus) dan bahan yang merangsang lebih larut dalam pelarut polar.

INTRODUCTION

Comfrey (*Symphytum officinale* Linn) was popular as "all disease" remedy in Indonesia until the Government stopped the circulation of the simplicium in the market due to lack of beneficial proof.

Symphytum officinale Linn cultivated in Indonesia is a member of the Family Boraginaceae.[1]) As a member of this Family it might contain pyrrolizidine alkaloids such as that found in foreign coutries like Europe and Australia or elsewhere. These alkaloids which are also found in the Senecio plants is said to produce contractions of the uterus.[2]) Due to the possible presence of this alkaloid the comfrey found in Indonesia might stimulate the uterus.[1]) The possible danger of the use of the material especially in pregnant women made this preliminary test on the isolated uterus necessary.

The infusion and extractions from the leaves of *Symphytum officinale* Linn obtained from the Balai Penelitian Tanaman Obat Tawangmangu in Surakarta (Central Java, Indonesia) were tested on the isolated with stillbestrol premedicated virgin guinea pig uterus. The experiment also showed the different affinity of the active part to solvents of different polarity.

MATERIALS AND METHODS

Leaves of *Symphytum officinale* Linn were obtained from the Balai Penelitian Tanaman Obat Tawangmangu in Surakarta, Central Java, Indonesia, and identified in the laboratory of Taxonomy of Plants in the Herbarium Bogoriensis in Bogor, Indonesia.

The dried leaves (dried at temperature not over 50° C) were powdered until the particles had a size of 48 Mesh.

Infusion was prepared according to the Indonesian Pharmacopoeia IInd edition.[3]) To obtain an infusion of higher concentration more material was used. To see the affinity of the active part, extracts were made by using solvents of different polarity along the scheme as shown below. The material was first macerated with 50 % aethanol and after evaporation at low pressure and low temparature the product was designated as *Fraction I*. NH_4OH was added to this fraction up to a pH of 10, and shaked with chloroform. The chloroform soluble part was designated as *Fraction II* and the chloroform *insoluble* part as *Fraction III*. This fraction was shaked with petroleum ether and the soluble part designated as *Fraction V*, the *insoluble*

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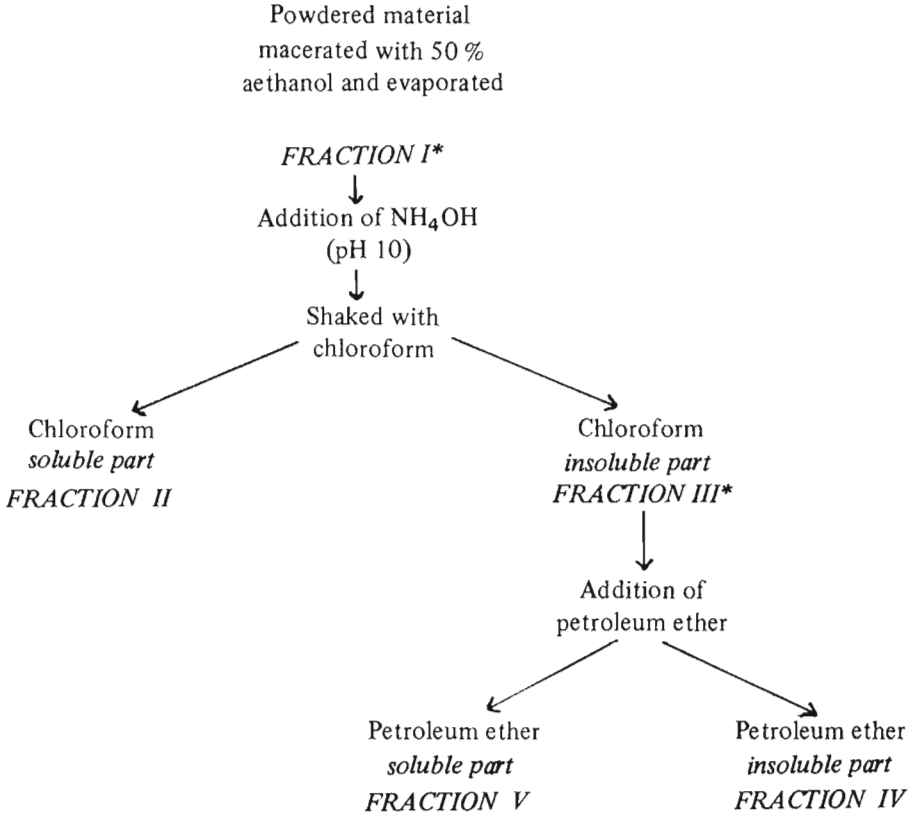
part as *Fraction IV*. All the fractions were tested on the isolated uterus. By recording the volumes of the extracts and taking into consideration the pre-extracted material, it was possible to estimate the equivalence of the fractions expressed in the powdered leaves. In this way the potency of the fractions can be estimated and compared.

The influence of the infusion and the extracts were tested on the isolated uterus according to H.J. Wilkens and R. Seger[4]) after several modifications. The modifications were: 1) air was used for oxygenation instead of 95 % oxygen, 2) Tyrode was used as the isotonic bath and 3) with stillbestrol pre-treated virgin guinea pig uterus was used instead of the rat uterus.

RESULTS

At dosages of 0.5 ml and 1.0 ml all the material tested stimulated the uterus. It was seen that the larger the dosage the larger the stimulation. *Fraction I, III and IV* caused the highest degree of stimulation, while *Fraction II and V* were less potent (see table 1).

SCHEME OF ISOLATION



* Part of each fraction is used for testing

Table 1. Relative potency of the infusion and extractions of *Symphytum officinale* Linn expressed in mm deflection

EXP NO DOSE IN ML MATERIAL TESTED	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III	
	0.5 ml	1 ml	0.5 ml	1 ml	0.5 ml	1 ml
Infusion 20%	1.25 ± 0.43	5.25 ± 0.83	1.25 ± 0.43	6.00 ± 1.41	1.33 ± 0.43	5.00 ± 0.71
Infusion 30%	2.00 ± 0.71	8.25 ± 0.83	2.75 ± 0.83	8.50 ± 0.50	1.50 ± 0.50	8.75 ± 0.83
FRACTION I	11.25 ± 1.63	13.00 ± 2.73	11.00 ± 1.73	13.25 ± 0.83	10.50 ± 1.12	14.75 ± 1.48
FRACTION II	2.00 ± 0.71	3.75 ± 1.48	1.25 ± 0.43	2.25 ± 0.83	1.00 ± 0.71	2.75 ± 0.83
FRACTION III	16.50 ± 0.86	18.25 ± 2.48	14.25 ± 1.48	16.00 ± 2.00	13.00 ± 1.58	15.75 ± 0.83
FRACTION IV	11.50 ± 2.69	15.00 ± 1.58	10.25 ± 0.43	12.25 ± 1.92	10.00 ± 1.41	12.50 ± 1.12
FRACTION V	4.25 ± 1.09	3.50 ± 1.12	3.50 ± 1.12	4.75 ± 0.83	1.75 ± 0.83	4.25 ± 1.64
Syntocinon	3.75 ± 0.83	8.00 ± 2.00	5.25 ± 1.78	8.50 ± 1.12	5.00 ± 0.70	10.25 ± 0.83

Tabel 2. Effect of the infusion and extractions of *Symphytum officinale Linn* at experimental dose, calculated human dose and potency ratio.

Material	Effect at experimental dose		Calculated effect at usual human dose (in mm deflection	Potency ratio *
	Dose in mg powder	Deflection in mm		
Infusion 30%	300	8.55	8.55	0.73
Fraction I	3000	12.83	1.28	0.11
Fraction II	3000	2.92	0.29	0.02
Fraction III	3000	16.00	1.6	0.14
Fraction IV	3000	13.25	1.32	0.11
Fraction V	3000	4.16	0.41	0.04
Syntocinon	0.02 IU	4.66	11.65	1

* Ratio of potency = $\frac{\text{deflection by material}}{\text{deflection by syntocinon}}$ (e.g. for 30 % infusion $\frac{8.55}{11.65} = 0.73$)

Three hundred mg of powder in the form of infusion stimulated the uterus up to 8.55 mm, while 3000 mg in the form of *Fraction I, II, III, IV and V* stimulated the uterus up to 12.8; 2.92; 16; 13.25; 4.16 mm respectively (see table 2). Assuming 0.02 IU of syntocynon as the usual dose causing contraction up to 11.65 mm, the relative potency of the infusion and the fractions are respectively 8.55; 1.28; 0.29; 1.60; 1.32; 0.41 (see table 2). It seems that even though *Fraction II and V* were still stimulating the uterus in a lower degree, they contain some inhibitory material, so that *Fraction IV and III* were more potent. Besides the infusion had the highest potency.

DISCUSSION

What we have obtained in the experiments on the isolated uterus is not equal to the results of the experiments in whole animals, especially when the test material must be administered orally. Any kind of changes can occur to the material before it could reach and act on the uterus after oral administration.

Besides, experiments on animals can not al-

ways be adopted directly to human beings, due to the difference of species and difference in their physiology. In view thereof, since the findings of this experiment, though making use of a standard material, gave some indication on the possible danger of the herb for human beings, further investigations are needed.

From the results it was seen that the infusion and all the fractions had a stimulating effect on the isolated uterus. It also seemed that by extraction an inhibitory factor could be separated from *Fraction I,* so that *Fraction II and V* were less potent. And by extraction it was also shwon, that the active part had a greater affinity to solvents with higher polarity. Therefore it is understandable that the danger of the empiric use of *Symphytum officinale* Linn leaves is great.

Even though the active part was more soluble in solvents with higher polarity it is still not proven whether the alkaloids, in this case pyrrolizidine alkaloids in the *Symphytum officinale* Linn, are the responsible material for the stimulating effect on the isolated uterus.

From the findings above, taking into consideration the potency of the infusion, it is advised not to use empirically at least for pregnant women. Anyhow the experiment gives support to the restriction of the circulation of the material in the market.

The *Symphytum officinale* Linn leaves for animal fodder, which was once introduced abroad and in Indonesia (personal communication),([5]) is also not recommended based on the experiment above.

Further experiments are needed to find out the active principle which stimulates the uterus and other possible toxic elements.

ACKNOWLEDGEMENTS

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LEGEND OF PLATES

Plate I 0.1 and 0.2 Syntocinon 0.02 and 0.1 IU resp.
a.1 and a.2 Aquadest 0.5 and 1 ml resp.
b.1 and b.2 Infusion 20 % 0.5 and 1 ml resp.
c.1 and c.2 Infusion 30 % 0.5 and 1 ml resp.
p = speed (p = 1 minute).

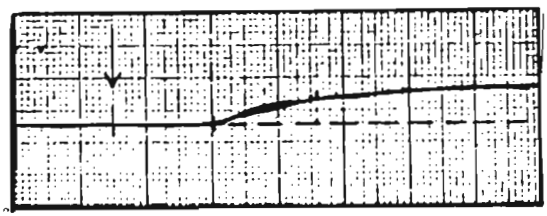
Plate II a.1 and a.2 Alkohol 50 % 0.5 and 1 ml resp.
b.1 and b.2 *Fraction I* 1.5 and 3 gram resp.
c.1 and c.2 Chloroform 0.5 and 1 ml resp.
d.1 and d.2 *Fraction II* 1.5 and 3 gram resp.
p = speed (p = 1 minute).

Plate III a.1 and a.2 Petroleum ether 0.5 and 1 ml resp.
b.1 and b.2 *Fraction V* 1.5 and 3 gram resp.
c.1 and c.2 *Fraction IV* 1.5 and 3 gram resp.
d.1 and d.2 *Fraction III* 1.5 and 3 gram resp.

Notice

1. The solvent aquadest, alkohol 50 %, chloroform and petroleum ether at dosages 0.5 and 1 ml/50 ml Tyrode had no effect whatsoever
2. The higher the dosage the larger the effect (see effect of infusion, Fraction I, Fraction II, Fraction III, Fraction IV and Fraction V)
3. The effect of infusion, Fraction I, Fraction IV and Fraction III, is larger than that of Fraction II and Fraction V. (the effect seen in the graph for the infusion must be multiplied by 10).

Plate 1



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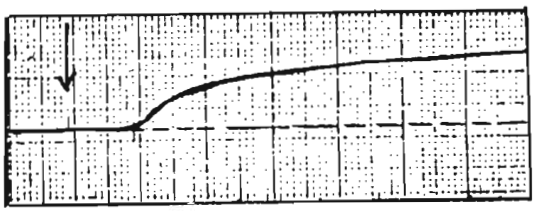

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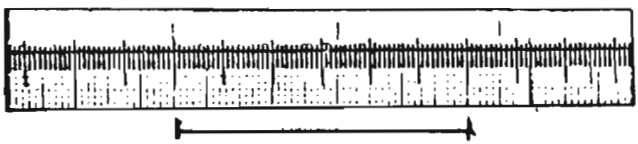

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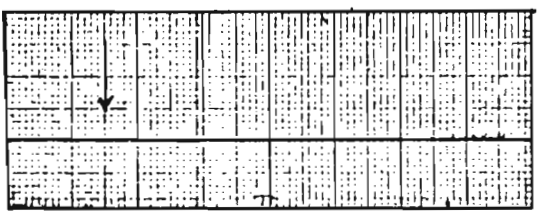

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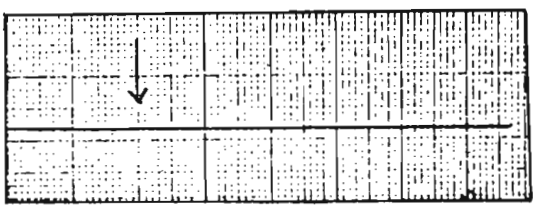

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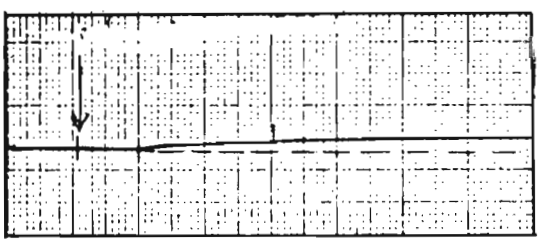

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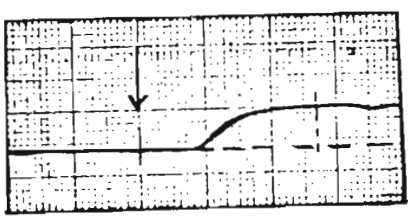

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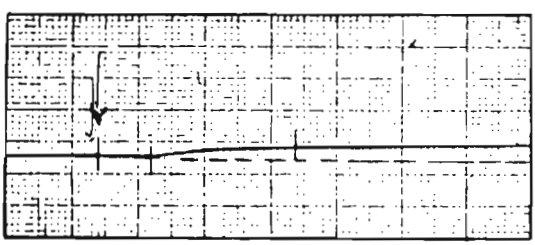

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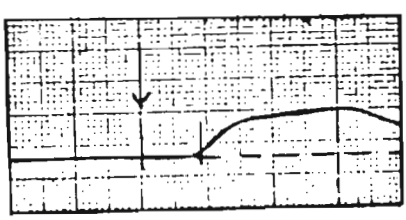

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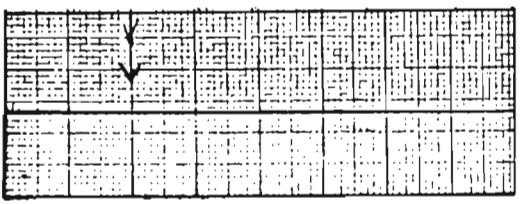

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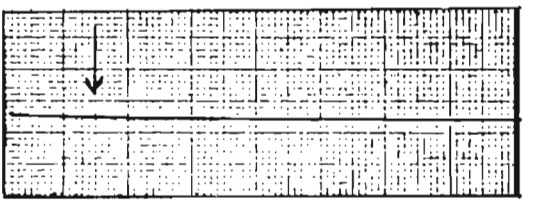

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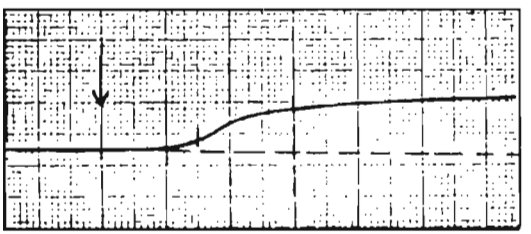

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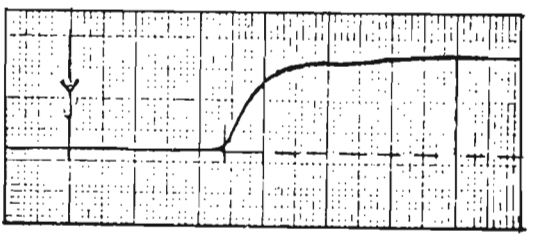

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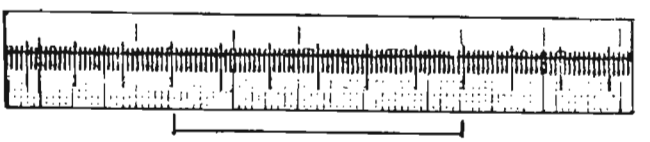

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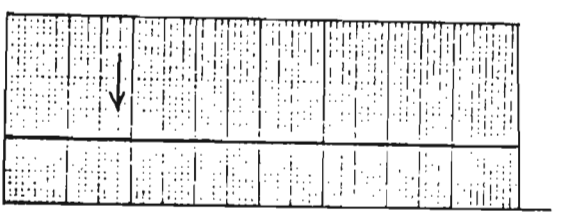

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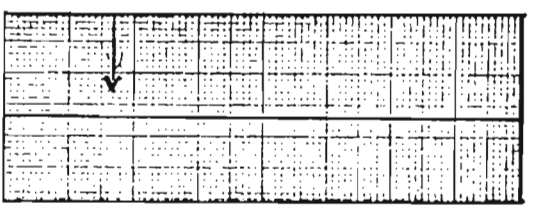

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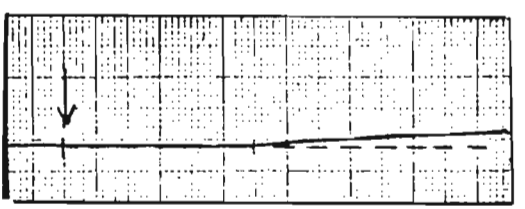

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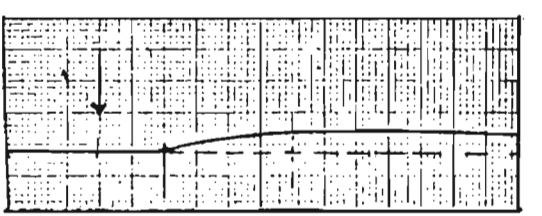

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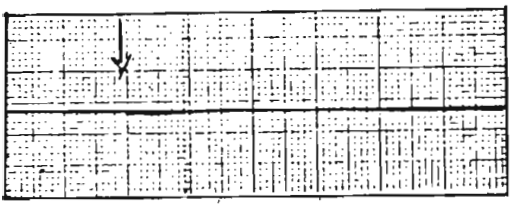

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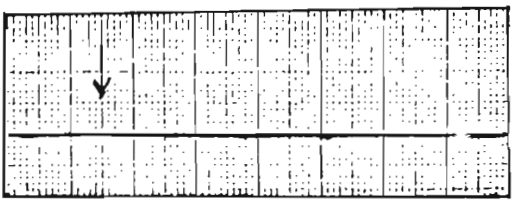

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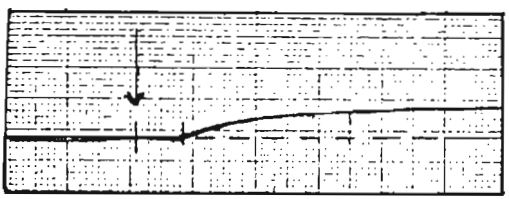

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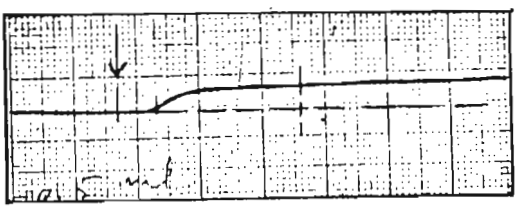

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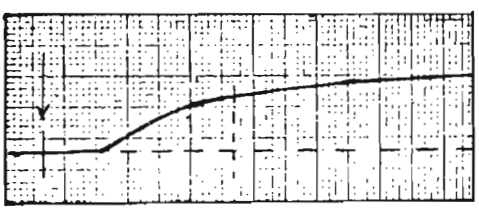

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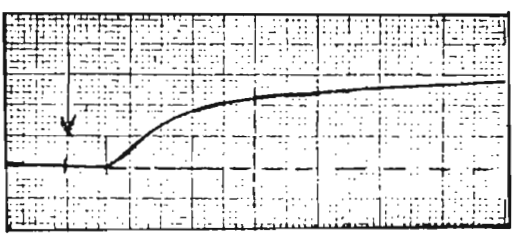

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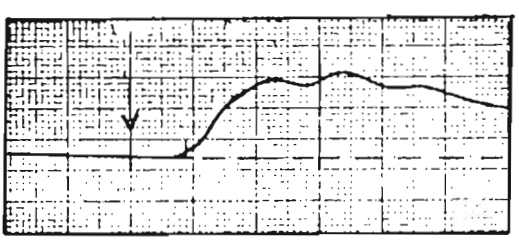

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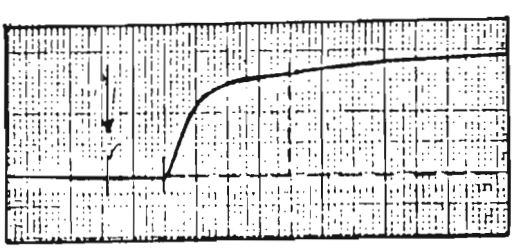

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